S8.P11

Supercomplex III/IV from hyperthermophilic eubacterium *Aquifex aeolicus*

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Respiratory supercomplexes have been extensively studied in mitochondria, but much less concerned in prokaryotes. Here we present the detailed characterization work on a supercomplex III/IV isolated from native membranes of eubacterium *Aquifex aeolicus*. This supercomplex III/IV survives from detergent solubilization and is monodispersed in both ion-exchange and size-exclusion chromatography. It consists of cytochrome b, cytochrome c1 and Rieske protein from the cytochrome bc1 complex and subunits I, II and IIa from the cytochrome oxidase. Major subunits are clearly visible on SDS-PAGE and later on identified by MALDI-MS. Activity assay showed that this supercomplex has higher turnover numbers than the purified cytochrome oxidase, which was called "Cox2" in our former work [1]. The stoichiometry of the supercomplex has been characterized as a homo-dimeric bc1 complex and a monomeric oxidase based on heme ratios, protein amounts and metal contents. Functional analysis on the supercomplex III2IV indicates potential interaction between the cytochrome bc1 complex and the cytochrome oxidase. In order to investigate the potential interaction, crosslinking work of the supercomplex has been performed, antibodies against protein subunits have been produced, and mass spectrometry has been applied. Further functional analysis of the supercomplex would be done as soon as possible aiming to get a picture of the supercomplex functional organization.

Reference

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S8.P12

Structural mapping of semiquinone catalytic intermediates at the quinol binding site of the nitrate reductase A from *Escherichia coli* by selective 15N labeling and pulsed EPR

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Isoprenoid quinones are liposoluble electron and proton carriers used in the vast majority of prokaryotic bioenergetic chains. Classified in either low or high redox potentials, quinones react with bioenergetic enzymes at specific quinone processing sites or Q sites. Deciphering the chemical processes catalyzed by Q-sites is challenging especially when these sites couple two one-electron transfer steps to the net release or uptake of two protons, as it occurs in dissociable Q sites. Indeed, this requires resolving the different binding modes of quinone, quinol and of the reactive semiquinone (SQ) intermediate. We use *Escherichia coli* nitrate reductase A (NarGHI) as a model enzyme to understand the role of the protein environment in tuning enzyme reactivity towards quinones of low (i.e. menaquinones, MK) and high (i.e. ubiquinones, UQ) redox potential. NarGHI is a membrane-bound heterotrimeric enzyme that couples the oxidation of quinols at a periplasmically-oriented Q site (named QD) to the cytoplasmic reduction of nitrate into nitrite [1]. Most interestingly, this complex was shown to stabilize the semiquinone catalytic intermediate of both MK and UQ [2]. High resolution pulsed EPR spectroscopy was used to explore the local environment of the protein-bound radicals with the use of NarGHI-enriched inner membrane vesicles containing endogenous quinones [2–4]. To directly assign the previously detected 14N-SQ interactions to specific nitrogen nuclei in the QD site [2,3], we have developed a selective 15N labeling strategy that relies on the construction and use of auxotrophic *E. coli* strains towards specific amino acids. This original approach allowed us for the first time to specifically probe the previously proposed role of nitrogen-containing residues in SQ binding [2,4] and thus to refine our current model of SQ binding mode in NarGHI [4]. Overall, our experimental strategy combining genetics, biochemistry, site-directed mutagenesis and most advanced EPR spectroscopies allowed us to attain an unprecedented level of structural understanding of distinct semiquinone binding at a single quinol site.

References

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S8.P13

The aerobic respiratory chain of the extremely acidophilic iron-oxidizing archaeon *Ferroplasma acidiphilum*

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Iron oxidation pathway by microorganisms is an important component of the iron biogeochemical cycle. Organisms able to oxidize ferrous iron to ferric iron are ubiquitous and affect various wide environments such as pyrite ores. Most of them are acidophilic bacteria or archaea and have an optimum pH for growth less than pH 3. The extremely acidophilic archaeon *Ferroplasma acidiphilum*, using ferrous iron as electron donor for aerobic growth, is widespread in sulfide ore deposits and is probably one of the major players in the biogeochemical cycling of sulfur and sulfides in highly acidic environments. Thus far, very little is known about the respiratory chains of this archaeal iron-oxidizer. Lacking genomic information, we here combine biochemical techniques such as spectroscopy, enzyme activities, purification and proteomic analysis to characterize the iron oxidation and oxygen reduction. We isolate two high molecular weight complexes that may function in the so-called uphill and downhill electron flows. The 850 kDa supramolecular complex contains an aa3-type oxidase and a